REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

AD-A253 904

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE

3. REPORT TYPE AND DATES COVERED
FINAL 15 May 89 TO 14 May 92

4. TITLE AND SUBTITLE
SYNAPTIC PLASTICITY AND MEMORY FORMATION

5. FUNDING NUMBERS
AFOSR-89-0383
PE 61102F
PR 2312
TA A2

(2)

6. AUTHOR(S)
Gary Lynch

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Center for the Neurobiology of Learning and Memory
Bonney Center
University of California
Irvine, CA 92717-3800

8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- 92 0743

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Dr Haddad
AFOSR/NL
Building 410
Bolling AFB DC 20332-6448

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

DTIC
ELECTE
AUG 12 1992
S A D

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;
distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Work conducted during AFOSR-89-0383 indicates that long-term potentiation is induced and stabilized by variants of the chemistries that regulate adhesive relationships. Expression of the potentiation effect involves modification of a subgroup of post-synaptic receptors; this modification includes a change in the kinetics of the receptor's ion channel. New evidence linking long-term potentiation to memory was obtained during the tenure of the grant and phamacological agents that promote its occurrence were identified. Based on this information, attempts to design and synthesize memory enhancing drugs have been initiated. The following paragraphs briefly describe these developments and cite relevant publications; a fuller description is found in the application for continuation of AFOSR support.

92 8 7 082

92-22448

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(U)

Final Technical Report for AFOSR grant 89-0383
"Synaptic Plasticity and Memory Formation"
Principal Investigator: Gary Lynch

Summary: Work conducted during AFOSR 89-0383 indicates that long-term potentiation is induced and stabilized by variants of the chemistries that regulate adhesive relationships. Expression of the potentiation effect involves modification of a subgroup of post-synaptic receptors; this modification includes a change in the kinetics of the receptor's ion channel. New evidence linking long-term potentiation to memory was obtained during the tenure of the grant and pharmacological agents that promote its occurrence were identified. Based on this information, attempts to design and synthesize memory enhancing drugs have been initiated. The following paragraphs briefly describe these developments and cite relevant publications; a fuller description is found in the application for continuation of AFOSR support.

The signal transmitted at synapses in the brain can be adjusted within seconds to a higher intensity by applying a brief sequence of high-frequency pulses in a specific pattern which mimics endogenous brain activity as it typically occurs during learning. The duration of this "long-term potentiation" (LTP) depends on the particular circuitry but can in some brain areas last for months or longer. There is considerable evidence that LTP encodes some forms of memory in forebrain networks and a description of its cellular bases should therefore be of considerable theoretical and practical significance.

Significant progress has been made during the last grant period (AFOSR 89-0383) in defining the processes responsible for induction, expression, and stabilization of LTP. Most importantly, several independent lines of research have provided converging evidence that the principle locus of the stable change resides on the postsynaptic side, more specifically in the AMPA type glutamate receptor which mediates fast synaptic transmission (**4, 7, 8, 28, 29**). LTP was found to alter a waveform parameter (the decay time constant or decay tau) directly related to the mean open time of the AMPA receptor (**34**). Changing the size of individual synaptic EPSPs did not affect the waveform of the responses and the LTP associated change in decay tau was obtained in slices from immature brains which have primitive spines and dendrites. These results confirm that the distortion in waveform produced by LTP is due to a modification of the kinetics of the voltage independent AMPA receptor channel. Further insight into the nature of the change was obtained with the drug aniracetam which, as shown in an AFOSR sponsored experiment, prolongs the mean open time of the AMPA receptor channel (**33**). The effects of aniracetam on the amplitude and shape of synaptic responses were substantially modified by LTP (but not by enhanced release) in a manner expected if potentiation alters the kinetics of the AMPA receptor channel (**23, 30, 38**). It should be noted that the waveform and pharmacological data accord well with the earlier discovery that LTP differentially affects response components mediated by AMPA receptors vs. the co-localized NMDA receptors (see previous Progress Reports). By themselves, the observed modifications would not enhance post-synaptic currents and so must occur as part of a receptor configuration that *in toto* involves channel kinetics and a shift in the conductance or affinity. Tests for a change in receptor affinity proved negative (**31**), pointing to a conductance increase as the agent of LTP expression. However, another group using a different autoradiographic technique has

reported that LTP increased binding to the AMPA receptor (43). The discrepancies between their results and ours need to be examined but it is likely that LTP expression is due to a change in receptor conformation that affects ligand affinity, channel conductance, and channel kinetics.

As indicated above, one can distinguish several phases in the formation of stable LTP. The process is set in motion by activation of NMDA receptors. Antagonist of this receptor were found to cause a substantial impairment of olfactory discrimination learning in rats (1); this finding has contributed to the evidence that LTP is a process subserving learning. In *in vitro* tests, the activity of the NMDA receptor can be modulated by a number of agents acting through several receptor sites. We have focused in our studies on one of these sites which binds glycine with high affinity (2, 5) and have shown that its activation is essential for receptor function inasmuch as its occupation by a selective antagonist prevented long-term potentiation (10, 11, 17). In a further approach, activation of NMDA receptors with exogenously added agonists was shown to produce a long-lasting enhancement of synaptic responses (6); whether the characteristics of this form of potentiation share similarities with those of LTP remains to be determined.

Various forms of evidence suggest that potentiation develops within 30 seconds of the induction. Once established, however, it appears to remain vulnerable to disruption for a period of minutes. This was shown in experiments in which a transient application of adenosine or a brief exposure to oxygen-free atmosphere followed the induction of LTP (12, 21). In both cases, the responses, after being transiently suppressed, returned to the level at which they had been before LTP induction. These findings indicate that the changes in the synaptic domain which account for LTP have to be consolidated over a period of about five minutes. Several biochemical mechanisms have now been demonstrated to be essential components of this process. One of them involves the activation of a receptor for platelet-activating factor (PAF)(22, 35); LTP induced in the presence of inhibitors of this receptor slowly decays as the responses return back to their pre-induction level. The PAF system in many peripheral cells aids in mobilizing calcium to orchestrate cellular changes and it is likely that it serves a similar function in the remodeling of the synapse during LTP. The effects of calcium are at least in part mediated by calpain, a calcium-sensitive protease, which is widely distributed in nerve cells including their dendrites (13) and has as its preferred targets a variety of structural proteins whose function it is to stabilize cell morphology; LTP elicited in presence of a calpain inhibitors consistently decayed back to baseline (9, 19). These observations have led to the idea that LTP involves a structural remodeling of the synapse in which calpain presumably would first degrade some of the existing structural components (e.g. spectrin) which then would lead the synaptic zone to reassemble in a new configuration. The processes involved in re-organization of the synaptic domain do not by themselves explain how the emergent configuration becomes stabilized. During the tenure of AFOSR 89-0383, we obtained evidence that transmembrane adhesion receptors belonging to the integrin family play a critical role in anchoring LTP. Specifically, small peptides that compete for the extracellular attachment site of a subgroup of integrins were found to prevent stabilization of potentiation (15, 32). Biochemical work then led to the purification of a protein greatly enriched in synaptic membranes that possesses the essential characteristics of an integrin including recognition by appropriate antibodies (24, 26, 39). Some integrins outside the brain are known to be latent in that they become adhesive only after an activation event; it is possible that the newly discovered synaptic integrin-like receptors

belong to this group. Thus, the re-forming of adhesion between adjacent cells, and presumably between pre- and postsynaptic elements, may be an essential part of the synaptic remodeling. We also obtained evidence that at least some of the proteins mediating cell adhesion are substrates of calpain (27).

As indicated above, the AMPA receptor is now thought to be the critical element in the expression of long-term potentiation. For this reason, efforts have been made to identify mechanisms which may control its functional properties. One conclusion from these studies has been that receptor affinity can be selectively altered after treating the membranes in which the receptors are embedded with certain phospholipases or supplementing them with phospholipids (14, 25). It is not unlikely that this is relevant for receptor function in intact cells as phospholipase inhibitors disrupted the stabilization of LTP (20). In another study we have found evidence that the AMPA receptor preferentially exists in two distinct affinity states and that certain manipulations such as the dissolution of the synaptic environment promote a conversion from the low into the high affinity state (41). The successful reconstitution of partially purified AMPA receptors (42) should allow us now to correlate these affinity states with functional properties of the receptor associated ion channel.

The above results provide a reasonably detailed, though in some aspects still tentative, picture of the events that induce, express, and stabilize a form of synaptic potentiation that is linked to the encoding of memories. It should now be possible to develop principled explanations for some forms of amnesia and design biologically based approaches to enhancing learning. Some steps in this direction were taken during the tenure of AFOSR 89-0383. Certain benzodiazepines used clinically as anxiolytics produce a profound anterograde amnesia. These drugs were found to be potent inhibitors of LTP (37), most probably via subtle effects on GABA receptors. Analyses of this possibility are now in progress. Other work directed at enhancing LTP focussed on the events occurring during and between the short bursts of afferent activity used to induce the potentiation effect. Greater depolarization during a burst reduced the number of bursts needed to produce maximal potentiation while suppression of the between-burst hyperpolarization elevated the ceiling on maximal LTP (40). The drug aniracetam, which as noted prolongs AMPA currents, emerged from these studies as a selective tool for enhancing LTP formation. The drug is not sufficiently potent and metabolically stable to be useful in this regard following peripheral administration and this led to attempts to synthesize variants that could be used in behavioral experiments. Substantial progress has been made in this area as described in previous Progress Reports.

Journal articles supported by AFOSR grant 89-0383 (1989 - 1992)

1. Staubli, U., Thibault, O., DiLorenzo, M. and Lynch, G. Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. Behav. Neurosci. **103**: 54-60, 1989.

2. Kessler, M., Baudry, M. and Lynch, G. Quinoxaline derivatives are high affinity antagonists of the NMDA receptor associated glycine sites. Brain Res. **489**: 377-382, 1989.

3. Muller, D. and Lynch, G. Rate-limiting step for transmission at excitatory synapses in hippocampus. Synapse **3**: 67-73, 1989.

4. Muller, D. and Lynch, G. Evidence that changes in presynaptic calcium currents are not responsible for long-term potentiation in hippocampus. Brain Res. **479**: 290-299, 1989.

5. Kessler, M., Terramani, T., Lynch, G. and Baudry, M. A glycine site associated with NMDA receptors: Characterization and identification of a new class of antagonists. J. Neurochem. **52**: 1319-1328, 1989.

6. Thibault, O., Joly, M., Muller, D., Schottler, F., Dudek, S., and Lynch, G. Long-lasting physiological effects of bath applied N-methyl-D-aspartate. Brain Res. **476**: 170-173, 1989.

7. Muller, D., Larson, J. and Lynch, G. The NMDA receptor mediated components of responses evoked by patterned stimulation are not increased by long-term potentiation. Brain Res. **477**: 396-399, 1989.

8. Muller, D., Oliver, M., and Lynch, G. Developmental changes of synaptic properties in hippocampus of neonatal rats. Developmental Brain Res. **49**: 105-114, 1989.

9. Oliver, M., Baudry, M. and Lynch, G. The protease inhibitor leupeptin interferes with the development of LTP in hippocampal slices. Brain Res. **505**: 233-238, 1990.

10. Oliver, M., Kessler, M., Larson, J., Schottler, F. and Lynch, G. Glycine site associated with the NMDA receptor modulates long-term potentiation. Synapse **5**: 265-270, 1990.

11. Oliver, M., Larson, J., and Lynch, G. Activation of the glycine site associated with the NMDA receptor is required for induction of LTP in neonatal hippocampus. Int. J. Dev. Neurosci. **8**: 417-424, 1990.

12. Arai, A., Larson, J., and Lynch, G. Anoxia reveals a vulnerable period in the development of long-term potentiation. Brain Res. **511**: 353-357, 1990.

13. Perlmutter, L.S., Gall, C., Baudry, M., and Lynch, G. Distribution of calcium-activated protease calpain in rat brain. J. Comp. Neurol. **296**:.269-276, 1990.

14. Massicotte, G., Kessler, M., Lynch, G. and Baudry, M. N-methyl-D-aspartate and quisqualate/AMPA receptors: Differential regulation by phospholipase C treatment. Mol. Pharmacol. **37**: 278-285, 1990.

15. Staubli, U., Vanderklish, P. and Lynch, G. An inhibitor of integrin receptors blocks long-term potentiation. Behav. Neural Biol. **53**: 1-5, 1990.

16. Muller, D., Buchs, P-A., Dunant, Y., and Lynch, G. Protein kinase C activity is not responsible for the expression of long-term potentiation in hippocampus. Proc. Natl. Acad. Sci. (USA) **87**: 4073-4077, 1990.

17. Oliver, M.W., Shacklock, J.A., Kessler, M., Lynch, G. and Baimbridge, K.G. The glycine site modulates the NMDA-mediated increase in intracellular free calcium from hippocampal cultures. Neurosci. Lett. **114**: 197-202, 1990.

18. Muller, D. and Lynch, G. Synaptic modulation of N-Methyl-D-Aspartate receptor mediated responses in hippocampus. Synapse **5**: 94-103, 1990.

19. del Cerro, S., Larson, J., Oliver, M.W., and Lynch, G. Development of hippocampal long-term potentiation is reduced by recently introduced calpain inhibitors. Brain Res **530**: 91-95, 1990.

20. Massicotte, G., Oliver, M.W., Lynch, G., and Baudry, M. Effect of bromophenacyl bromide, a phospholipase A_2 inhibitor, on the induction and maintenance of LTP in hippocampal slices. Brain Res. **537**: 49-53, 1990.

21. Aral, A., Kessler, M., and Lynch, G. The effects of adenosine on the development of long-term potentiation. Neurosci. Lett. **119**: 41-44, 1990.

22. del Cerro, S., Aral, A., and Lynch, G. Inhibition of long-term potentiation by an antagonist of platelet-activating factor receptors. Behav. Neural Biol. **54**: 213-217, 1990.

23. Staubli, U., Kessler, M. and Lynch, G. Aniracetam has proportionately smaller effects on synapses expressing long-term potentiation: Evidence that receptor changes subserve LTP. Psychobiology **18**: 377-381, 1990.

24. Bahr, B.A., Sheppard, A., Vanderklish, P.W., Bakus, B.L., Capoldi, D. and Lynch, G. Antibodies to the $\alpha_v\beta_3$ integrin label a protein concentrated in brain synaptosomal membranes. NeuroReport **2**: 321-324, 1991.

25. Massicotte, G., Vanderklish, P., Lynch, G., and Baudry, M. Modulation of DL-alpha-amino-3- hydroxy-5-methyl-4-isoxazolepropionic acid-quisqualate receptors by phospholipase-A_2: A necessary step in long-term potentiation. Proc. Natl. Acad. Sci. (USA) **88**: 1893-1897, 1991.

26. Bahr, B.A., Sheppard, A., and Lynch, G. Fibronectin binding by brain synaptosomal membranes may not involve conventional integrins. NeuroReport **2**: 13-16, 1991.

27. Sheppard, A., Wu, J., Rutishauser, U., and Lynch, G. Proteolytic modification of neural cell adhesion molecule (N-CAM) by the intracellular proteinase calpain. Biochim. Biophys. Acta **1076**: 156-160, 1991.

28. Jung, M.W., Larson, J. and Lynch, G. Evidence that changes in spine neck resistance are not responsible for expression of LTP. Synapse **7**: 216-220, 1991.

29. Larson, J. and Lynch, G. A test of the spine resistance hypothesis of LTP expression. Brain Res. **538**: 347-350, 1991.

30. Xiao, P., Staubli, U., Kessler, M., and Lynch, G. Selective effects of aniracetam across receptor types and forms of synaptic facilitation in hippocampus. Hippocampus **1**: 373-390, 1991.

31. Kessler, M., Arai, A., Vanderklish, P., and Lynch, G. Failure to detect changes in AMPA receptor binding after long term potentiation. Brain Res. **560**: 337-341, 1991.

32. Xiao, P., Bahr, B.A., Staubli, U., Vanderklish, P.W., and Lynch, G. Evidence that matrix recognition contributes to the stabilization but not induction of LTP. NeuroReport **2**: 461-464, 1991.

33. Tang, C-M., Shi, Q-Y., Katchman, A., and Lynch, G. Modulation of the time course of fast EPSCs and glutamate channel kinetics by aniracetam. Science **254**: 288-290, 1991.

34. Ambros-Ingerson, J., Larson, J., Xiao, P., and Lynch, G. LTP changes the waveform of synaptic responses. Synapse **9**: 314-316, 1991.

35. Arai, A. and Lynch, G. Antagonists of the platelet-activating factor receptor block long-term potentiation in hippocampal slices. Eur. J. Neurosci. **4**: 411-419, 1992.

36. Muller, D., Arai, A., and Lynch, G. Factors governing the potentiation of NMDA receptor-mediated responses in hippocampus. Hippocampus **2**: 29-38, 1992.

37. del Cerro, S., Jung, M., and Lynch, G. Benzodiazepines block long-term potentiation in rat hippocampal and piriform cortex slices. Neurosci. **49**: 1-6, 1992.

38. Staubli, U., Ambros-Ingerson, J., and Lynch, G. Receptor changes and LTP: An analysis using aniracetam, a drug that reversibly modifies glutamate (AMPA) receptors. Hippocampus **2**: 49-58, 1992.

39. Bahr, B.A. and Lynch, G. Purification of an ARG-GLY-ASP selective matrix receptor from brain synaptic plasma membranes. Biochem. J. **281**: 137-142, 1992.

40. Arai, A. and Lynch, G. Factors regulating the magnitude of LTP induced by theta pattern stimulation. Brain Res. (in press).

41. Hall, R.A., Kessler, M., and Lynch, G. Evidence that high and low affinity AMPA binding sites reflect membrane-dependent states of a single receptor. J. Neurochem. (in press).

42. Bahr, B.A., Vodyanoy, V., Hall, R., Suppiramiam, V., Kessler, M., Sumikawa, K., and Lynch, G. Functional reconstitution of AMPA-type glutamate receptors from rat brain. J. Neurochem. (submitted).

Other cited publications:

43. Tocco, G., Maren, S., Shors, T.J., Baudry, M., and Thompson, R.F. Long-term potentiation is associated with increased [^{3}H]AMPA binding in rat hippocampus. Brain Res. **573**: 228-234, 1992.